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A new species of *Alainites* (Ephemeroptera, Baetidae) from Thailand

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Abstract

A new species of Baetidae, *Alainites siamensis* **sp. nov.**, is described from Thailand. This new species is closely related to *Alainites lingulatus* Tong & Dudgeon, 2000, *Alainites laetificus* (Kang & Yang, 1994) and *Alainites yixiani* (Gui & Lu, 1999). Species delimitation based on morphological and molecular (mitochondrial COI sequences) evidence is provided. The discovery of this species confirms the wide distribution of *Alainites* Waltz & McCafferty, 1994 in Southeast Asia.

Key Words

distribution, diversity, mayflies, Southeast Asia, systematic

Introduction

The small minnow mayflies (Ephemeroptera: Baetidae) are one of the most common and widespread mayfly family. This family comprises ca. 1,070 species assigned to 110 genera (Sartori and Brittain 2015; Jacobus et al. 2019; Kaltenbach et al. 2020).

The European species of *Baetis* s.l. Leach, 1815 were firstly classified and divided into eleven species groups by Müller-Liebenau (1969). The *muticus* species group was subsequently raised to the generic level under *Alainites* Waltz & McCafferty, 1994 (Waltz et al. 1994; McCafferty and Waltz 1997; Zrelli et al. 2012; Yanai et al. 2022). *Nigrobaetis* Novikova & Kluge, 1987 was erected for the *niger* species group (Waltz et al. 1994). Finally, *Takobia* Novikova & Kluge, 1987 was established and encompassed a single species originally described as *Centroptilum maxillare* Braasch & Soldán 1983.

Alainites, *Nigrobaetis* and *Takobia* were considered as belonging to the *Indobaetis* complex (Kluge and Novikova 2014). The status of the three genera was widely debated in various studies (Müller-Liebenau 1969; Novikova and Kluge 1987, 1994; Waltz et al. 1994; Waltz and McCafferty 1997; Kluge and Novikova 2014). However, the revisions were not based on examination of type material and detailed descriptions of the type species were missing (see details in Sroka et al. 2021; Yanai et al. 2022). In the last comprehensive studies (Sroka et al. 2021; Yanai et al. 2022), it was highly encouraged to continue to consider *Alainites*, *Nigrobaetis* and *Takobia* as valid genera and wait for a global phylogeny based on molecular and morphological evidence before proposing a definitive classification.

The genus *Alainites* originally encompassed nine species (Waltz et al. 1994); at that time the diagnostic characters of the larval stage were as follows: i) paraproct with an elongated prolongation; ii) prostheca of the right mandible bifid, reduced to two bristle-like feathered appendages; iii) absence of villopore; and iv) body laterally compressed. At the imaginal stages: i) hindwings, when present, with three longitudinal veins, the second being bifurcated; and ii) segment III of the male forceps spherical to slightly elongated and curved (Waltz et al. 1994; Zrelli et al. 2012; Yanai et al. 2022).

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In the Oriental realm, seven species were assigned to *Alainites*. Part of these species were originally attributed to *Baetis*, including *Baetis laetificus* Müller-Liebenau, 1984, *Baetis* (*Acerbaetis*) *clivosus* Kang & Yang, 1994 and *Baetis* (*Acerbaetis*) *yehi* Kang & Yang, 1994. Afterward, these three *Baetis* species were transferred to *Alainites* by Waltz et al. (1994). The Chinese species, *Alainites yixinia* (Gui & Lu, 1999) was also first attributed to *Baetis* (Gui and Lu 1999). Two new species of *Alainites* from Hong Kong, *Alainites acutulus* Tong & Dudgeon, 2000 and *Alainites lingulatus* Tong & Dudgeon, 2000, were subsequently described (Tong and Dudgeon 2000). The last described species of *Alainites* in South East Asia, *Alainites pascalae* Gattolliat, 2011, was reported from Borneo (Gattolliat 2011).

The knowledge of the diversity of Baetidae in Thailand has notably increased recently. Thanks to two large scale surveys of the Baetidae in Thailand project many taxa have been continuously discovered during the last decade (Tungpairojwong and Bae 2015; Phlai-ngam and Tungpairojwong 2018; Suttinun et al. 2018, 2020, 2021, 2022; Phlai-ngam et al. 2022; Tunjpairojwong et al. 2022). Many new taxa have been reported, some of them remain undescribed. Presently, ten genera and 14 species of Baetidae are recorded from this area (Phlai-ngam 2017; Suttinun 2021; Suttinun et al. 2021, 2022; Boonsoong 2022; Phlai-ngam et al. 2022; Tungpairojwong et al. 2022). Based on the remaining under prospected areas and the still unstudied but potentially diversified genera (*Baetis*, *Labiobaetis* Novikova & Kluge, 1987, *Nigrobaetis*), the species diversity of Thai Baetidae will continue to increase rapidly.

Herein, we provide the description and illustration of larval stage of a new species of *Alainites* from Thailand. The morphological comparison of this new species to related species, provides diagnostic key characters. The species delimitation is also supported by molecular evidence (mitochondrial COI sequences). Additionally, a key to species of the larvae of *Alainites* from Southeast Asia is also provided.

Materials and methods

Collecting samples

Larval specimens were collected by using a hand net or picked manually and sorted from the debris and sediments by using D-framed dip net method. This new species was collected for the first time during a survey of aquatic macroinvertebrates in Phetchabun Province and the survey of aquatic macroinvertebrates project of Kanchanaburi Province (Thailand). The additional specimens were collected during the survey of baetid mayflies in Tak and Kamphaengphet Provinces (Table 1). They were sampled in headwater streams. The specimens were preserved in 95% ethanol. The examined material is deposited in the Collection of Aquatic Insect of Department of Biology at Khon Kaen University in Khon Kaen, Thailand **(KKU-AIC)** and in the Museum of Zoology in Lausanne, Switzerland **(MZL)**.

Table 1. GPS coordinates of locations of examined specimens.

Species	Provinces	GPS coordinates	Altitudes (m a.s.l.)
A. siamensis **sp. nov.**	Phetchabun	16°44'27.92"N, 101°34'46.52"E	832
	Tak	17°04'52.68"N, 98°45'16.73"E	405
		17°01'44.35"N, 98°30'24.47"E	719
	Kamphaengphet	17°02'34.94"N, 98°58'39.83"E	154
	Kanchanaburi	14°45'08.00"N, 98°48'40.00"E	660

Morphological examination

Part of the specimens were dissected and mounted on microscope slides fixed in Euparal, as specified in the material examined sections below. Ethanol-preserved specimens were studied under a Leica M205 stereomicroscope; microscope slides were drawn from a camera lucida on an Olympus BX51 compound microscope and were subsequently scanned for illustration with the Procreate application (iOS application). Photographs of larvae were taken with a Canon EOS 6D camera and edited with Adobe Lightroom (http://www.adobe.com). Final plates were prepared and processed with Adobe Photoshop (http://www.adobe.com). The distribution map was generated with the SimpleMappr software (https://simplemappr.net).

Genetics

DNA of part of the specimens was extracted using non-destructive methods allowing subsequent morphological analysis (see details in Vuataz et al. 2011). The specimens were amplified for a 658 bp fragment of the mitochondrial gene cytochrome oxidase subunit 1 (COI) using the primers LCO 1490 (GGTCAACAAATCATAAAGATATTGG) and HCO 2198 (TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al. 1994).

The polymerase chain reaction (PCR) was conducted with an initial denaturation temperature of 94 °C for 5 min followed by a total of 35 cycles with denaturation temperature of 94 °C for 30 sec, an annealing temperature of 48 °C for 40 sec and an extension at 72 °C for 1 min, final extension at 72 °C for 5 min. The sequencing was based the Sanger's method as developed in Vuataz et al. (2011). Sequences editing and ClustalW alignment were provided. Genetic variability between specimens was calculated using Kimura-2-parameter distances (K2P) model. The molecular reconstruction was analyzed by a maximum likelihood (ML). The best evolution model obtained was Tamura-Nei (TN93+G+I) as the most appropriate for reconstruction based on the lowest AICc and BIC scores, with 100 runs and 1000 bootstrap replicates. All genetic analytical methods were performed by MEGA-X (Kumar et al. 2018). Additional *Alainites* sequences were obtained from GenBank (http://www.ncbi.nlm.nih.gov/) and new *Alainites* sequences (this work) were also added in GenBank to update the nucleotides database (Table 2).

Table 2. Sequenced specimens of *Alainites* (bold text showing new sequences).

Species	Locality	GenBank Accession Number (GenSeq Nomenclature)
A. talasi	Kyrgyzstan	MZ983799.1; MZ983800.1
A. sadati	Algeria	ON072439
A. gasithi	Israel	ON072440
A. kars	Armenia	MZ983797.1; MZ983798.1
A. bengunn	Sardinia	HG934996.1; HG934997.1
A. albinatii	Corsica	HG934994.1
A. yixiani	China	GU479735.1
A. muticus	Spain	SR10E08
	Russia	SR13E10
***A. siamensis* sp. nov. (PHET1)**	Phetchabun, Thailand	**OP903356**
***A. siamensis* sp. nov. (PHET2)**	Phetchabun, Thailand	**OP903357**
***A. siamensis* sp. nov. (PHET3)**	Phetchabun, Thailand	**OP903358**
***A. siamensis* sp. nov. (TAK1)**	Tak, Thailand	**OP903355**
***A. siamensis* sp. nov. (TAK2)**	Tak, Thailand	**OP903359**
***A. siamensis* sp. nov. (KAMP)**	Kamphaengphet, Thailand	**OP903360**

Results

Alainites siamensis sp. nov.

https://zoobank.org/DF090CF3-0863-4454-A90B-EB58DFB6D49D

Material. ***Holotype*: Thailand** • Larva; Phetchabun Province, Nam Nao National Park, Yakruae stream; Alt. 832 m. 16°44'27.92"N, 101°34'46.52"E; 7 Mar. 2022; Coll. S. Phlai-ngam and V. Vannachak; 1L on slide GBIFCH00763744 [MZL].

***Paratypes*: Thailand** •20 larvae; same data as holotype; 3L on slides GBIFCH00763745–GBIFCH00763747, [MZL]; 10L in alcohol GBIFCH00763748 [MZL]; 7L in alcohol [KKU-AIC].

Other material. Thailand • 70 larvae; **Tak Province**, Mae Ra Mad District; Alt. 405 m. 17°04'52.68"N, 098°45'16.76"E; 12 Feb. 2022; Coll. K. Koomput and P. Dapsibhai; 1L on slides GBIFCH00763749 [MZL]; 8L in alcohol GBIFCH00763750 [MZL]; 10L in alcohol [KKU-AIC]. **Tak Province**, Mae Sod District, small stream near the road (unnamed stream); Alt. 719 m. 17°01'44.35"N, 098°30'24.47"E; 8 Oct. 2020; coll. S. Phlai-ngam; 1L on slide [KKU-AIC]; 14L in alcohol [KKU-AIC]; 1L in alcohol GBIFCH 00673245 [MZL]. **Kanchanaburi Province**, Thong Pha Phumi District, small stream near the Mining Dr. Phol Kleepbuathe; Alt. 660 m. 14°45'08.00"N, 98°48'40.00"E; 16 Nov. 2022; Coll. S. Phlai-ngam; 1L on slide GBIFCH00763751 [MZL]; 7L in alcohol GBIFCH00763752 [MZL], 8L in alcohol [KKU-AIC]. **Phetchabun Province**, Nam Nao National Park, Yakruae stream; Alt. 832 m. 16°44'27.92"N, 101°34'46.52"E; 23 Nov. 2020; Coll. S. Phlai-ngam; 15L in alcohol [KKU-AIC]. **Kamphaengphet Province**, Klong Lan District, Pong Nam Ron, Alt. 154 m. 17°02'34.94"N, 98°58'39.83"E; 12 Feb. 2022; Coll. K. Koomput and P. Dapsibhai; 4L in alcohol [KKU-AIC].

Description. ***Coloration*** (Figs 1, 2). General coloration medium brown. Head uniformly medium brown, slightly darker between ocelli and at insertion of antennae. Turbinate eyes in male larva dark orange. Thorax medium brown with indistinct pattern. Pronotum slightly paler than mesonotum and metanotum. Thoracic sternites mostly pale brown. Abdominal tergites medium brown, slightly darker in middle area, distal margin with darker transverse band. Abdominal sternites light brown. Cerci and median caudal filament light brown without bands or pattern.

Body. Maximum length 4.2 mm. Median caudal filament ca. 2/3 of cerci.

Head. Capsule medium brown. Antennae medium brown, filiform.

Mouthparts. Labrum (Fig. 3A): dorsal surface with one central seta and an arc of setae reduced to two lateral setae; about twenty fine stout setae scattered over surface. Ventral surface with five small acute setae near lateral margin. Distal margin fringed with two kinds of setae: long lateral setae strongly feather-like and medially with a shorter row of slightly feather-like setae.

Left mandible (Fig. 3B–D): inner and outer incisors almost fused, formed by seven blunt denticles (Fig. 3C); prostheca with eleven small pointed denticles apically, (Fig. 3D); edge between prostheca and mola only slightly crenelate near mola; mola with an enlarged triangular pointed tooth, fine setae present apically; proximal part with scattered fine setae, not shagreened (Fig. 3B). Right mandible (Fig. 3E–G): inner and outer incisors almost fused, formed by eight blunt denticles (Fig. 3F), prostheca bifid, both filaments feathered, outer filament half length inner filament (Fig. 3G); edge between prostheca and mola crenelate; proximal part with scattered fine setae, not shagreened (Fig. 3E). Maxilla (Fig. 3H–I): galea-lacinia with 3 enlarged acute teeth on apex (Fig. 3I), 2 rows of long dense basal setae on galea-lacinia; maxillary palpus (Fig. 3H) 2-segmented with scattered small hair-like setae; segment II rounded at apex. Labium (Fig. 3J): glossa slightly shorter than paraglossa; a row of stout setae along outer, inner and apical margins, subapical setal tuft present; paraglossae with 3 rows of simple setae along apical margin; labial palpus 3-segmented with scattered fine setae; segment I slightly shorter than segments II and III combined; segment III subrectangular covered with long robust setae mostly in distal half, short pointed setae mostly along outer margin and abundant fine setae. Hypopharynx with rounded lingua and superlinguae, covered with abundant hair-like setae subapically (Fig. 3K).

Thorax. Hindwing pads well developed.

Foreleg (Fig. 4A). Femur: dorsal margin with a regular row of eight to eleven long robust setae and two subapical setae; ventral margin with abundant short spine-like setae; lateral margin with scale bases, bare and not shagreened, femoral villopore absent.

Tibia (Fig. 4B): dorsal margin of fore-tibia with less than five strong setae (usually with 3–4 setae), short fine setae roughly arranged in row; ventral margin with

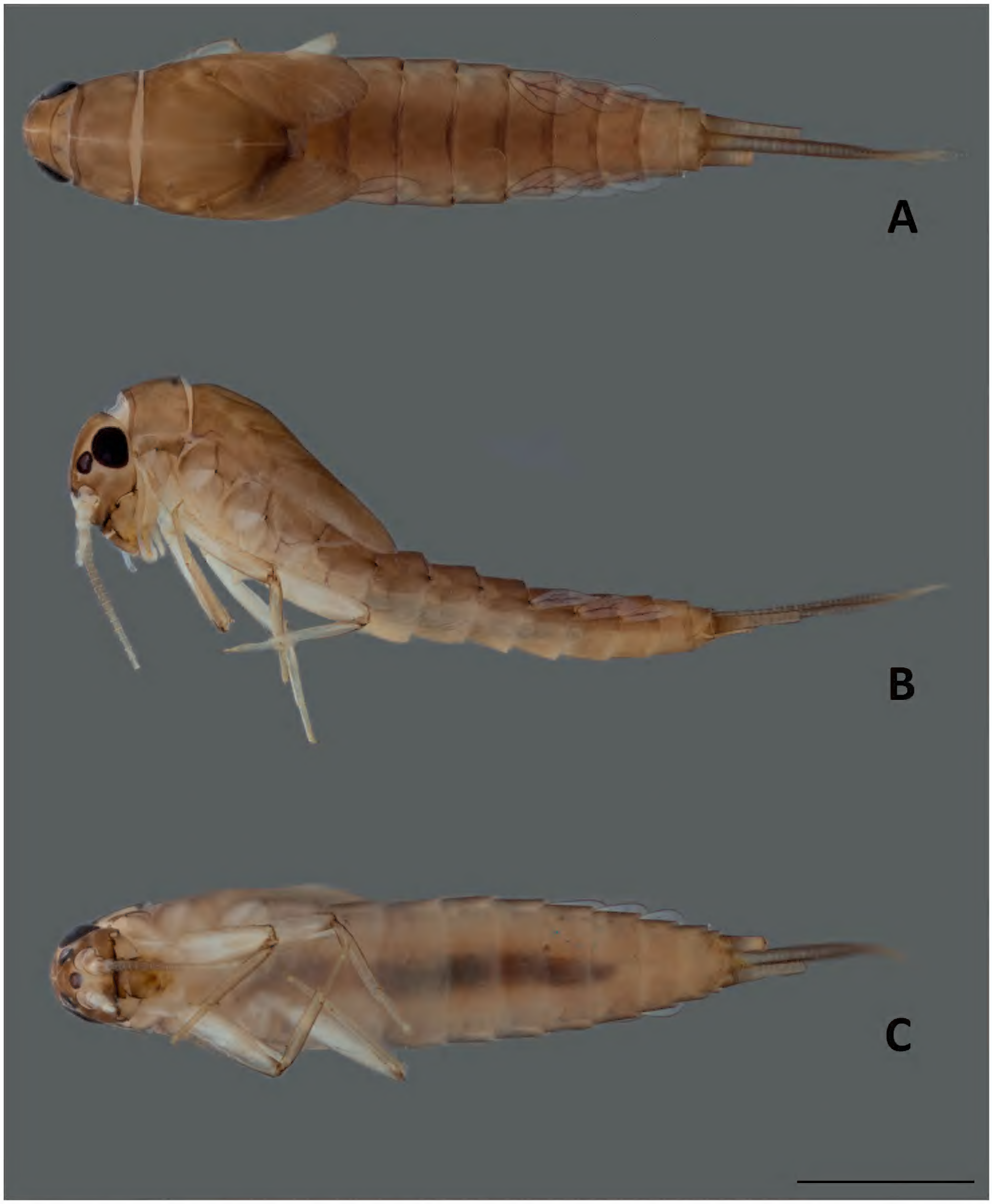

Figure 1. *Alainites siamensis* sp. nov., female larva (middle instar larva) habitus: **A** Dorsal view; **B** Lateral view; **C** Ventral view. Scale bar: 1 mm.

abundant short stout setae ending with patch of long stout feathered setae; lateral margin with abundant scale bases, tibiopatellar suture with short spine-like setae. Tarsus: dorsal margin with few fine setae, ventral margin with abundant pointed setae only slightly increasing in length toward apex, lateral margin with abundant scale bases and a few fine setae. Tarsal claws (Fig. 4E) with one row of 7–9 denticles, increasing in size distally, subapical setae absent. ***Midleg and hindleg.*** Similar to foreleg; except ventral margin of femur of hindleg with reduced number of short spine-like setae, generally one or two setae; dorsal margin of mid- tibia (Fig. 4C) and hind-tibia (Fig. 4D) with more than five strong setae (usually more than nine).

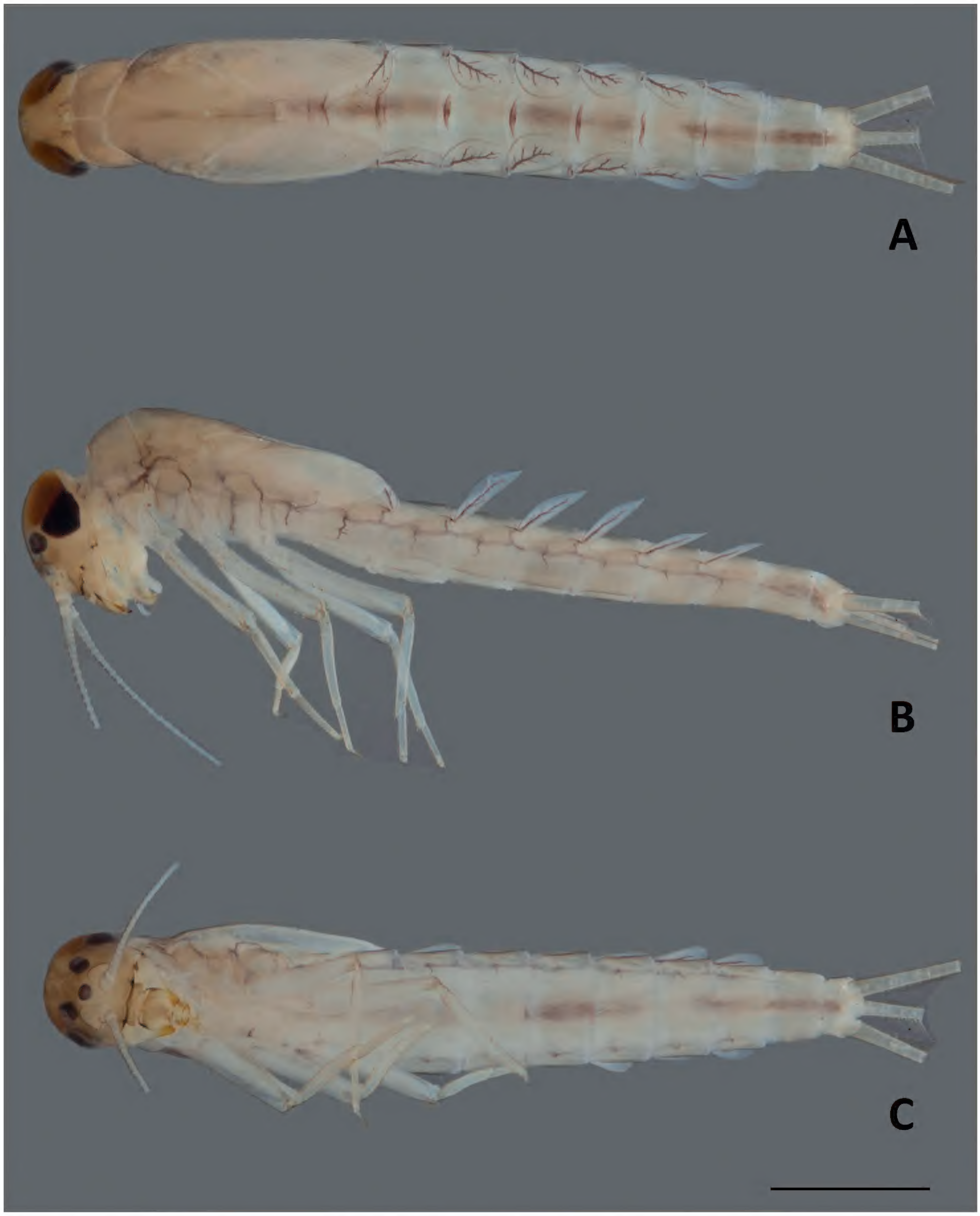

Figure 2. *Alainites siamensis* sp. nov., male larva (middle instar larva) habitus: **A** Dorsal view; **B** Lateral view; **C** Ventral view. Scale bar: 1 mm.

Abdomen. Posterior margin of tergite I smooth; posterior margin of tergite II with a few triangular spines mainly in the middle area; posterior margin of tergites III–IX with triangular spines along central portion, absent laterally (Fig. 4F–G). Surface of all tergites not shagreened, with numerous scale bases and few setae. Sternites I–VIII similar to tergites except distal margin smooth, sternite IX distal margin with triangular spines. Gills (Fig. 5A–D) on segments I–VII, with well visible main tracheation but reduced ramification; gill I smallest (Fig. 5A), length of gill I equal to half of tergite II; gill IV (Fig. 5B) and gill V larger than others, length of gill IV equal to tergite V plus half of

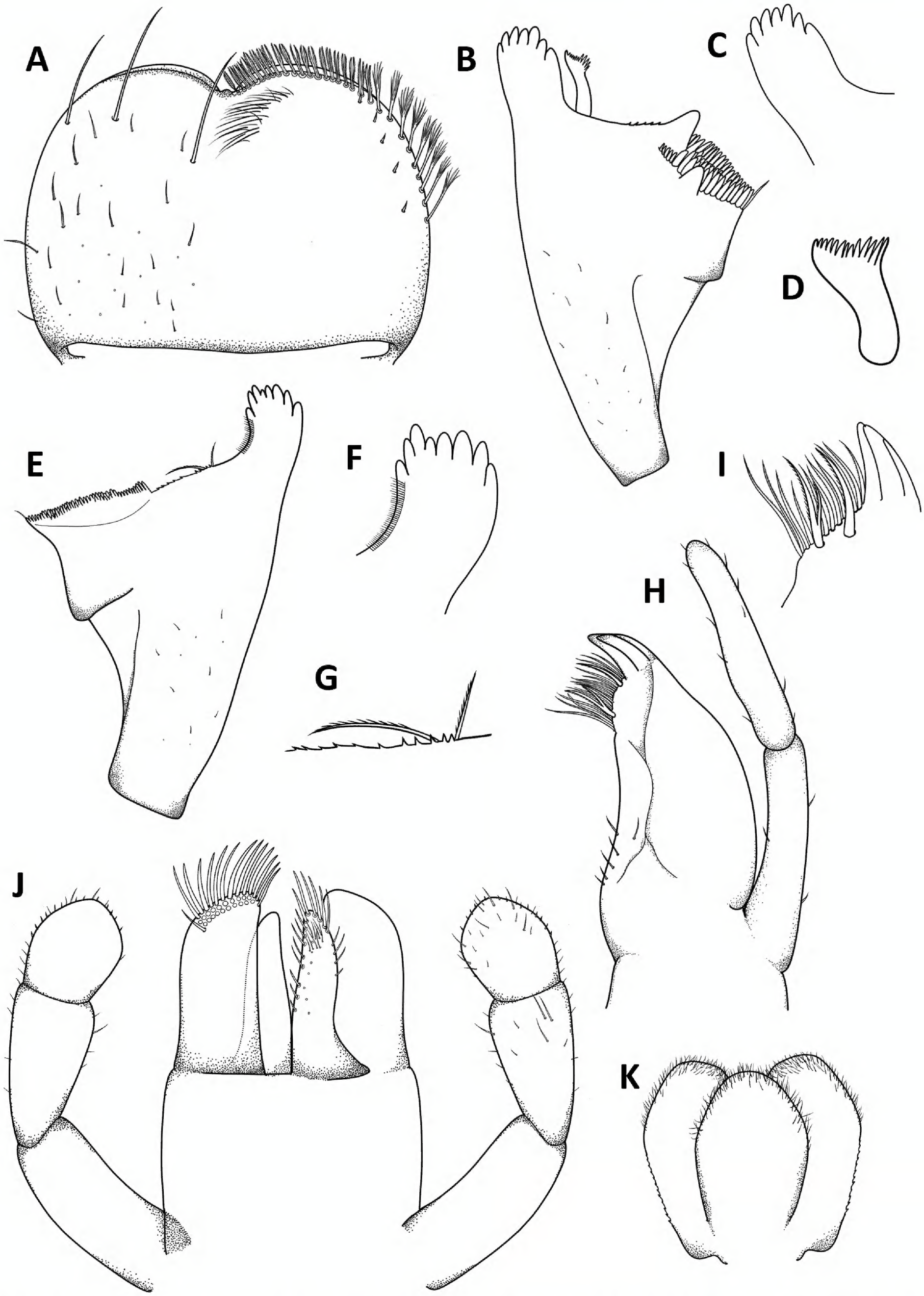

Figure 3. *Alainites siamensis* sp. nov., larval morphology: **A** Labrum (right: dorsal; left: ventral); **B** left mandible; **C** left incisor; **D** left prostheca; **E** Right mandible; **F** Right incisor; **G** Right prostheca; **H** Maxilla; **I** Apex of galea-lacinia; **J** Labium (right: dorsal; left: ventral); **K** Hypopharynx.

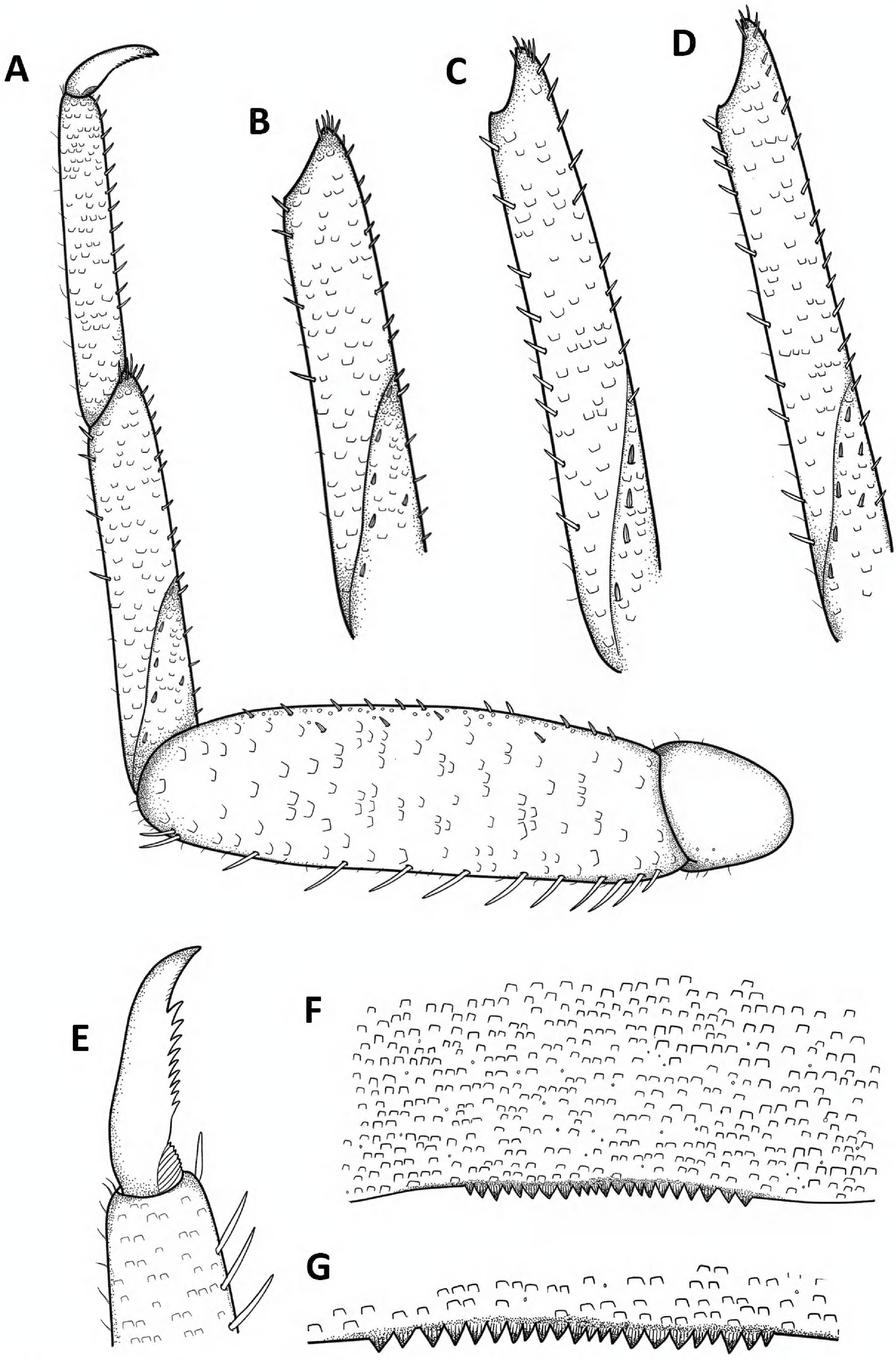

Figure 4. *Alainites siamensis* sp. nov., larval morphology: **A** Foreleg; **B** Fore-tibia; **C** Mid-tibia; **D** Hind-tibia; **E** Tarsal claw; **F** Abdominal tergite IV; **G** Posterior marginal spines of tergite IV.

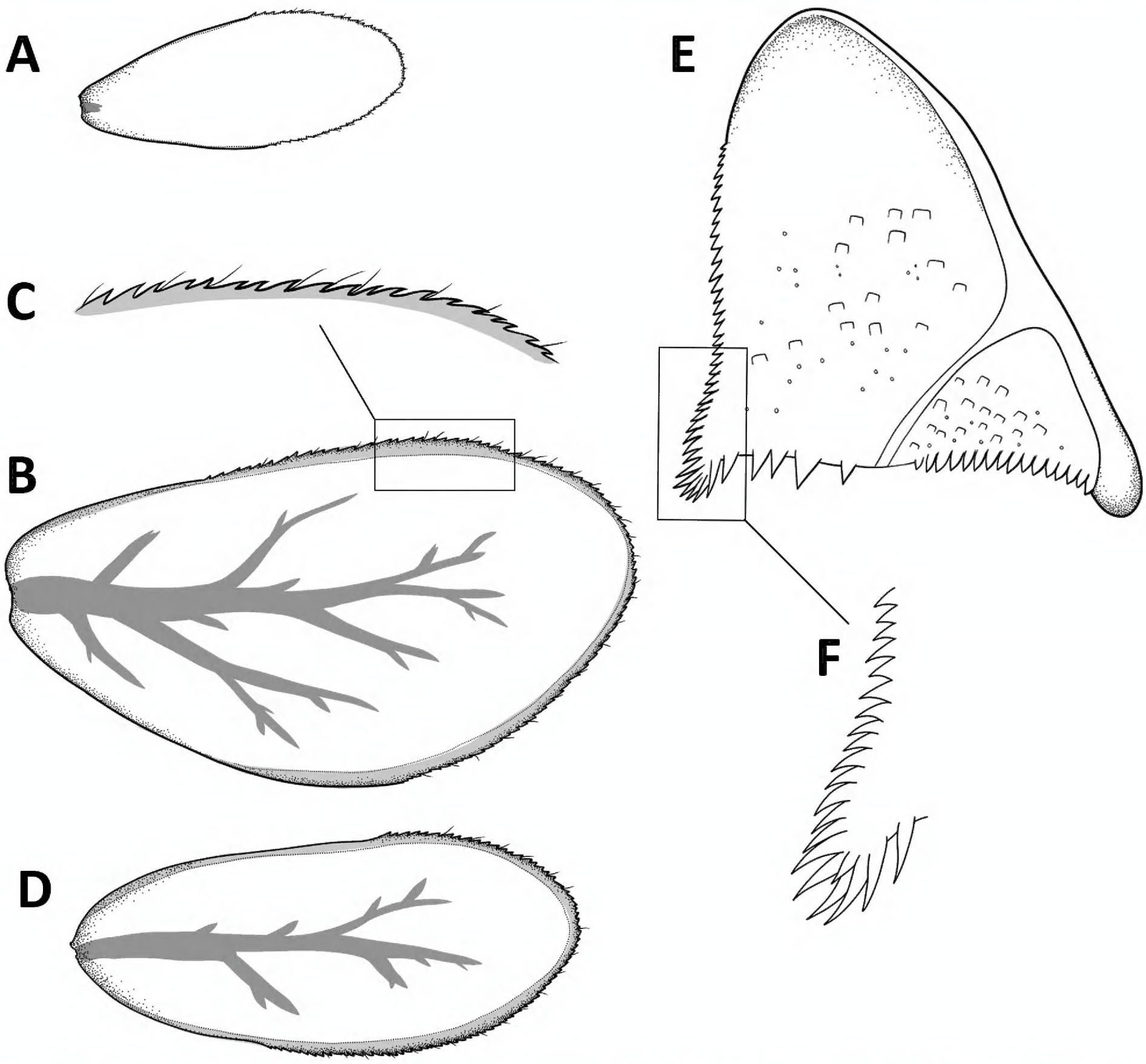

Figure 5. *Alainites siamensis* sp. nov., larval morphology: **A** Abdominal gill I; **B** Abdominal gill IV; **C** Gill margin; **D** Abdominal gill VII; **E** Paraproct; **F** Paraproct prolongation.

tergite VI; length of gill V equal to tergite VI plus half of tergite VII; length of gill VII (Fig. 5D) equal to tergite VIII plus half of tergite IX. Surface smooth without any setae or pores, margin serrated with fine simple setae (Fig. 5C). Paraproct (Fig. 5E–F) covered with scale bases and micropores on surface; distal margin with well-developed prolongation; lateral margins of prolongation with numerous small spines, surface without spines (Fig. 5F); distal margin inner to prolongation with 5 huge spines, distal margin outer to prolongation with numerous medium spines (around 20–30 spines); cercotractor with scale bases, distal margin with about 14–16 medium spines (Fig. 5E). Cerci with fine swimming setae along inner margin. Median filament with swimming setae on both margins.

Diagnosis. *Alainites siamensis* sp. nov. is closely related to *A. lingulatus*, *A. laetficus* and *A. yixiani*; these species are distributed in the Southeast Asia. The new species can be separated from the other species by the following combination of characters: i) the shape of labial palp, ii) the setation on tibia, iii) the number of denticles of the claws, iv) the degree of development and spination of paraproct prolongation, v) the number of spines on distal margin of paraproct, vi) number of pairs of gills (Table 4).

Ecological notes. The larvae of *Alainites siamensis* sp. nov. mainly live among aquatic plants and roots of riparian plants in slow to moderately flowing streams (Fig. 6). The species seems to be rather widely distributed in the northwestern and northern Thailand, but is rare and not abundant (Fig. 7).

Etymology. The specific epithet of this species, *siam* (noun), refers to the old name of Thailand and to the known distribution of the species.

Molecular result. Sequences of 658 bp, corresponding to a fragment of the COI gene, were obtained from

Figure 6. The sampling sites and habitats of *Alainites siamensis* sp. nov. larvae: **A–B** Yakruae stream, Nam Nao National Park, Phetchabun Province (type locality); **C–D** Stream in Mae Ra Mad District, Tak Province; **E–F** Stream near the Mining Dr. Phol Kleepbuathe, Thong Pha Phum District, Kanchanaburi Province.

six specimens collected in three localities; the new sequences are deposited in GenBank under accession numbers specified in Table 2. The K2P analysis for genetic distances analysis shows that the three populations of *Alainites siamensis* sp. nov. have very low intraspecific variation (0% to < 2%). The interspecific distances between *A. siamensis* sp. nov. and other *Alainites* species show distances between 17% and 28% (Table 3). In the COI reconstruction, the six larval specimens of *Alainites* from Thailand were grouped together into the same clade and clearly separated from all other sequenced species of *Alainites* (Fig. 9).

Discussion

The new described mayfly species from Thailand, *Alainites siamensis* sp. nov., is assigned to the

Figure 7. Distribution map of *Alainites siamensis* sp. nov. larvae: Closed red triangle shows the type locality, opened red triangle shown other localities.

Table 3. Genetic distances (COI) between sequenced specimens, using the Kimura 2-parameter.

		1	2	3	4	5	6	7	8	9
1	*A. siamensis* **sp. nov.**	0.00–0.02								
2	*A. yixiani*	0.20	-							
3	*A. albinatii*	0.21	0.21	-						
4	*A. kars*	0.24	0.20	0.20	0.00					
5	*A. talasi*	0.21	0.24	0.28	0.25	0.00				
6	*A. sadati*	0.22	0.24	0.23	0.23	0.25	-			
7	*A. bengunn*	0.26	0.26	0.20	0.25	0.27	0.24	0.01		
8	*A. gasithi*	0.22	0.23	0.23	0.19	0.24	0.24	0.22	0.00	
9	*A. muticus*	0.22	0.23	0.17	0.22	0.28	0.24	0.20	0.23	0.02

genus *Alainites* Waltz and McCafferty, 1994 based on the following characters: frons with a medial ridge between antenna, absence of distal lobe on antennal scape, prostheca of the right mandible bifid, absence of villopore, paraproct with prolongation (Waltz et al.1994; Fujitani et al. 2003; Zrelli et al. 2012; Yanai et al. 2022). This new species presents similarities with the type species of the genus, *Alainites muticus* (Linnaeus, 1758); both species possess seven pairs of gills and well-developed paraproct prolongation. The two species can be separated by inconspicuous characters such as the number of setae on dorsal margin of tibia, setation of distal margin and prolongation of paraproct. *Alainites muticus* is not reported from Southeast Asia and from the Oriental realm in general; the closest report seems to be in Korea (Bae and Park 1998).

Among the Southeast Asian species, *Alainites siamensis* sp. nov. can be identified by the shape of labial palp, the setation of tibia, the number of denticles of the claws, the degree of development and spination of paraproct prolongation, the number of spines on distal margin of paraproct and the number of pairs of gills (Table 4). The presence of seven pairs of gills in *Alainites siamensis* sp. nov. allow the separation of this species from *A. aculatus* Tong & Dudgeon, 2000, *A. laetificus* Müller-Liebenau, 1984, *A. pascalae* Gattolliat, 2011 and *A. yehi* (Kang & Yang, 1994) which all possess six pairs of gills.

The new species differs from the other species with seven pairs of gills (*A. lingulatus* Tong & Dudgeon, 2000, *A. clivosus* (Chang & Yang, 1994) and *A. yixiani* (Gui & Lu, 1999)) by presence of less than five strong setae on dorsal margin of foretibia (usually with 3–4 setae), tarsal claws with 7–9 denticles, well-developed paraproct prolongation, in opposition to *A. clivosus* and *A. yixiani* which possess more than five strong setae on dorsal margin of foretibia and tarsal claws with more than nine denticles. *Alainites clivosus* can be distinguished from *A. yixiani* by the abdominal tergites uniformly medium brown while *A. yixiani* has tergites IX and X lighter than the remaining tergites.

Table 4. Comparison of larval morphological characters of *Alainites siamensis* sp. nov. with the closely related Southeast Asian species with seven pairs of gills. Character states based on the original descriptions of individual species.

Characters	*A. clivosus*	*A. lingulatus*	*A. yixiani*	*A. siamensis* **sp. nov.**
Shape of terminal segment of labial palp	Sub-rectangular shaped; lateral margin slightly rounded	Sub-rectangular shaped; lateral margin almost straight	Sub-rectangular shaped; lateral margin slightly rounded	Sub-rectangular shaped; lateral margin almost straight
Setation of dorsal margin of foretibia	More than five strong setae	More than five strong setae	More than five strong setae	Less than five strong setae (usually 3–4 setae)
Number of denticles of the tarsal claws	11–13 denticles	8–11 denticles	9–11 denticles	7–9 denticles
Abdominal tergites	Uniformly medium brown	Uniformly medium brown in mature female; tergites I–VI light brown and tergites VII–X brown in mature male larvae	Tergites I–VIII uniformly medium brown; IX and X light brown	Uniformly medium brown
Distal margin of abdominal tergites	Tergite I smooth; tergites II–X with triangular spines along central portion, absent laterally	Tergite I smooth, tergites II–X with triangular spines	?	Tergite I smooth; tergite II with a few triangular spines mainly in the middle area; tergites III–IX with triangular spines along central portion, absent laterally
The degree of development of paraproct prolongation	Moderately developed	Well-developed tongue-like	Moderately developed	Well-developed
Distribution	Taiwan	Hong Kong	China mainland	Thailand
Reference	Kang et al. (1994)	Tong and Dudgeon (2000)	Gui and Lu (1999)	This work

Alainites siamensis sp. nov. can be distinguished from *A. lingulatus* by the shape of the prolongation of the paraproct and by claws with 7–9 denticles while *A. lingulatus* has paraproct with a well-developed tongue-like prolongation and claws with 8–11 denticles (Table 4).

Alainites laetificus and *A. pascalae* share with the new species the distal margin of tergite IV with spines and prolongation of paraproct well developed with numerous spines. *Alainites siamensis* can be easily separated from these two species by the number of gills.

The molecular analysis clearly confirms that *Alainites siamensis* sp. nov. is a valid new species as shown by genetic distances between species ranging from 17% to 28% (Table 3), which are greater than 3% generally considered as the maximum value for intraspecific divergence (Hebert et al. 2003). The intraspecific divergences are rather reduced, generally lower than 2%. The genetic distances were calculated between the eight available species, i.e. *A. talasi* (Novikova & Kluge, 1994), *A. sadati* Thomas, 1994, *A. gasithi* Yanai & Gattolliat, 2022, *A. kars* (Thomas & Kazanci, 1989), *A. bengunn* Yanai & Gattolliat, 2022, *A. albinatii* (Sartori & Tomas, 1989), *A. muticus* (Linnaeus, 1758), and *A. yixiani*. However, several sequences are missing for part of the *Alainites* species, especially for the morphologically closely related species *A. lingulatus* and *A. laetficus*. *Alainites yixiani* is the only sequenced species from Southeast Asia available for calculation.

The morphological comparison between species is often challenging by the short inaccurate original description and the poor quality of the illustrations; this remains true also for recently described species such as *A. yixiani*. A revision of this species is high request and will be necessary to confirm the characters isolated for species identification. However, the molecular analysis based on COI sequences confirm the distinctness of these two species (K2P distance = 20% between the two clades). A complete molecular data analysis of the Southeast Asian *Alainites* might take time for integrating all previous species reports from this area. We hope that, in a close future, integrative approach including molecular data, accurate descriptions and geographic distribution will help to securely identify the different species of *Alainites* in Southeast Asia.

Alainites is widely distributed in Southeast Asia, but the eight species are reported from a a single country (Fig. 8). Amazingly, five of the eight are only reported from islands. The new species, *Alainites siamensis* sp. nov. is only known from Thailand which represents a new report from an inland country in midway between the previous reports in Southeast Asia.

According to ecological requirement and the presumed dispersal abilities of *Alainites*, we may expect a wider distribution in Southeast Asia which includes most of inland countries of the area, as well as overseas colonization of some islands in particular in the Philippines, as supported by the presence of the genus in Taiwan, Borneo and Hong Kong (Müller-Liebenau 1984; Kang et al. 1994; Tong and Dudgeon 2000; Gattolliat 2011). As already mentioned, almost all species show restricted distribution and are only known from a single country. This is at least partially due to the lack of data in some areas. We can expect new reports from Cambodia, Laos, Myanmar (Burma), or Vietnam; they will almost certainly increase the distribution of part of the species or allow the discovery of new ones. A global molecular study of the different species will greatly help to understand the mechanism of dispersion and speciation within these taxa.

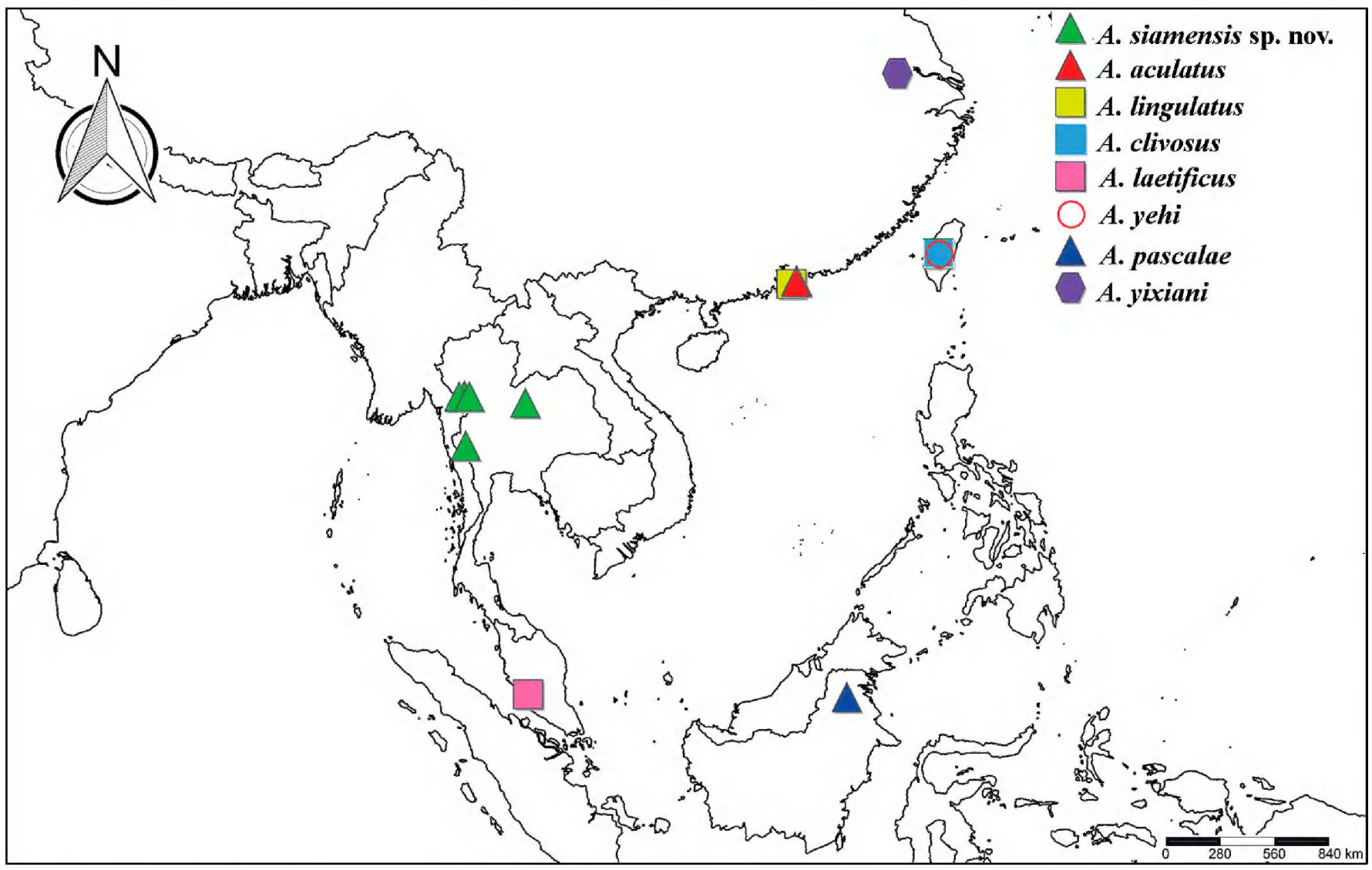

Figure 8. Distribution map of *Alainites* species in Southeast Asia.

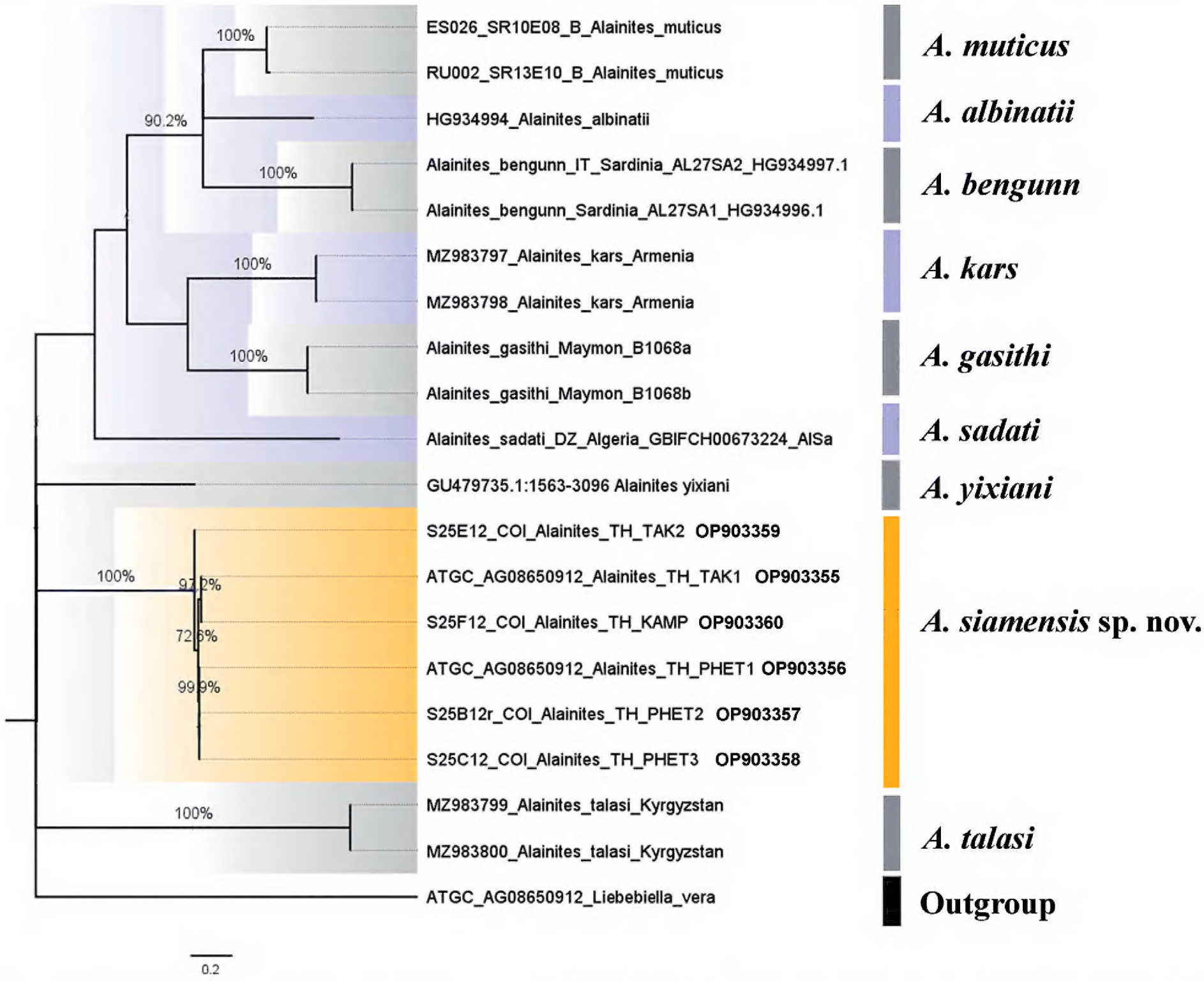

Figure 9. Phylogenetic reconstruction of *Alainites* species based on maximum likelihood analysis of sequences of the mitochondrial COI gene. The reconstruction includes representatives of the new species, *Alainites siamensis* sp. nov. (Orange background), other *Alainites* species (Blue and gray background) and *Liebebiella vera* (Black color) as outgroup.

Key to the larvae of *Alainites* from Southeast Asia*

1 7 pairs of gills..... 2
– 6 pairs of gills..... 5
2 Paraproct with a tongue-like prolongation, broader apically than medially.....*A. lingulatus*
– Paraproct with a moderately or well-developed prolongation, not tongue-like..... 3
3 Abdominal tergites uniformly medium brown..... 4
– Abdominal tergites I–VIII uniformly medium brown; tergites IX and X light brown ..... *A. yixiani*
4 Tarsal claws with 7–9 denticles..... *A. siamensis* sp. nov.
– Tarsal claws with 11–13 denticles..... *A. clivosus*
5 Distal margin of tergite IV with spines; prolongation of paraproct well developed with numerous spines..... 6
– Distal margin of tergite IV without spines; prolongation of paraproct moderately developed or well-developed tongue-like projection 7
6 More than ten strong setae on the dorsal margin of mid tibia; short spine-like setae between prostheca and mola ....*A. pascalae*
– Less than five strong setae on the dorsal margin of mid tibia; long spine-like setae between prostheca and mola ....*A. laetificus*
7 Paraproct with short, slightly acute prolongation (moderately developed); ventral margin of fore femora with robust, simple setae ..... *A. acutulus*
– Paraproct with well-developed tongue-like prolongation; ventral margin of fore femora without setae.....*A. yehi*

* Based on the original description and illustration.

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